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Primula sunhangii (Primulaceae): a new species from Hubei, Central China

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Abstract

This report provides a description of *Primula sunhangii* from the Shennongjia Forestry District, Hubei Province in Central China, which is categorized as a new species of the primrose family. *Primula sunhangii* is morphologically similar to *P. involucrata* Wall. ex Duby in terms of its simple umbel, efarinose, and prolonged bracts. However, *P. sunhangii* is distinguished by its glabrous sepal, short petiole (compare with blade) and cylindrical calyx and capsule. Molecular phylogenetic analysis based on nuclear and cpDNA genes demonstrates that *P. sunhangii* and *P. involucrata* are closely related. Combining genetic and morphological data, the recognition of *P. sunhangii* as a unique new species is supported.

Keywords

morphological and molecular data, new species, *Primula*, Shennongjia

* The authors contributed equally to this study.

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Introduction

The well-known horticultural genus *Primula* is the largest in the Primulaceae. There are approximately 500 *Primula* species worldwide, with the majority distributed in the North Temperate Zone and a small number of outlying species located in the mountainous regions of Africa (e.g. Ethiopia), tropical Asia (e.g. Java and Sumatra), and South America (Richards 2002). In general, *Primula* is distinguished from other genera by being multi-scapose with a long corolla and heterostylous flowers. There are approximately 300 native *Primula* species in China, for which the preferred habitat is the relatively warm and humid regions of the Himalayas and Hengduan mountainous regions, in particular the Yunnan and Sichuan provinces of Southwest China that represent *Primula* biodiversity hotspots (Hu and Kelso 1996).

Currently, many *Primula* species are poorly characterized, with a number of descriptions based on a single sample, which potentially results in undocumented details of characteristics such as pin or thrum morphology, fruiting, and ecology (Hu and Kelso 1996). Taxonomic study of *Primula* in China remains insufficient and requires continued efforts, as demonstrated by the large number of reports describing new species, including *P. pengzhouensis* C. M. Hu, G. Hao & Y. Xu. (Xu et al. 2017), *P. anthemifolia* G. Hao, C. M. Hu & Yuan Xu (Xu et al. 2015), and *P. mianyangensis* G. Hao & C. M. Hu (Wu et al. 2013) collected from Sichuan; *P. hubeiensis* X. W. Li (Li et al. 2017) collected from Hubei; and *P. undulifolia* G. Hao, C. M. Hu & Y. Xu (Xu et al. 2016) collected from Hunan.

The Shennongjia Forestry District, located in western Hubei, Central China, adjacent to eastern Sichuan, is well known for its rich biodiversity, in particular the rare and endangered golden monkey. The Shennongjia mountain range is a large geographical area (ca. 3,250 km^2) known as the roof of central China, which represents the second geographical step of China in a west-east direction and is characterized by the majority of its mountain peaks being over 3,000 m. The flora of this area is mainly influenced by the monsoon from the Pacific Ocean, which differs from the weather systems that affect Yunnan or western Sichuan in Southwest China. Moreover, its environment is moist from March to November, which is maintained by the local atmosphere, influenced by both the Yangtze and Hanjiang rivers that surround the Shennongjia area. (Deng et al. 2018)

In 2011, during several field expeditions in Shennongjia, an unusual *Primula* population was discovered, comprised of plants similar to *P. involucrata*, belonging to Sect. *Aleuritia* Duby, with comparable glabrous and efarinose leaves, denticulate leaf margins, umbels, cylindrical calyx, funnel-form corolla and bracts base prolonged below into auriculate appendage. However, these individuals were clearly distinguished by their glabrous sepal, cylindrical calyx and capsule, truncate or acute leaves base, and shorter scape. We thus hypothesized this population represents a new species, which was verified by subsequent morphological and molecular phylogenetic comparisons and thus described as *Primula sunhangii*.

Materials and methods

Morphological analysis

Morphological analysis was performed using recently collected specimens and those sampled in 2011 from Shennongjia. A vernier caliper and a dissecting microscope were used in the measurement. We referred to the keys to sections and species in Flora Reipublicae Popularis Sinicae (Hu 1990) and Flora of China (Hu and Kelso 1996). We also examined all species in Sect. *Aleuritia*. Specimens are deposited in the herbaria of Kunming Institute of Botany (**KUN**) and Jishou University (**JIU**).

Taxon sampling and outgroup selection

The phylogenetic analysis was mainly based on the recently published framework of *Primula* (Yan et al. 2015), with Sect. *Aleuritia* as the focal group. We performed sequencing of nuclear ITS as well as *rbcL* and *matK* of *P. sunhangii*, and downloaded 84 sequences of 28 species from GenBank. *Omphalogramma delavayi* (Franch.) Franch. was selected as the outgroup. All GenBank accession numbers of studied taxa are listed in Suppl. material 1.

DNA extraction and sequencing

Molecular materials were collected from Shennongjia in 2011. A Tiangen DNA extraction kit was used. PCR was performed according to standard protocols (Kusukawa et al. 1990). The primers used were those described by Yan et al. (2015) (Table 2). DNA sequencing was performed by Tsingke Biological Technology in Kunming.

Phylogenetic Analysis

The raw sequences were manually edited and aligned using BioEdit 7.0.4.1 (Hall 2001). In the ITS, *rbcL*, *matK* and combined (including all three markers above) phylogenetic analysis, maximum likelihood (ML) analysis was conducted using RAxML 8.2.10 (Stamatakis 2014) and run for 1,000 bootstrap iterations under a GTRGAMMA model. Bayesian inference (BI) analysis was carried out using MrBayes 3.2.6 (Ronquist et al. 2012) and run for 2,000,0000 generations under a GTR + I + G substitution model, which was selected using jModelTest2 2.1.6 (Darriba et al. 2012).

Taxonomic treatment

Primula sunhangii **T. Deng, D. G. Zhang & Jiao Sun, sp. nov.**
urn:lsid:ipni.org:names:77211168-1

Type. China, Hubei Province, Shennongjia Forestry District, Hongping, Dashuping. 31°26.67'N, 110°16.01'E Alt. 2877 m. 5 Jun 2011, D. G. Zhang et al. Zdg20110605023 (Holotype **KUN**!) (Fig. 1). – ***Isotype:*** China, Hubei Province, Shennongjia Forestry District, Hongping, Dashuping. 31°26.67'N, 110°16.01'E Alt. 2877 m. 5 Jun 2011, D. G. Zhang et al. Zdg20110605023 (**JIU**!).

Diagnosis. *Primula sunhangii* is similar to *P. involucrata* in glabrous, efarinose, ovate leaves, and lanceolate bracts base prolonged below into auriculate appendage and its length. But it differs from *P. involucrata* in the following characters: glabrous sepal, cylindrical calyx and capsule, truncate or acute leaves base, and shorter scape (at most 3 times the leaves).

Description. Herbs perennial, glabrous, efarinose. Leaves 5–10, all basal in a rosette; petiole green, basal white to pink, 1–5.6 cm long, 0.2–0.4 cm diam.; leaf blade green, ovate or oblong, 0.8–4.8 cm long, 0.5–2 cm wide, papery, base truncate or acute, margin entire or slightly denticulate, apex obtuse to rounded. Scapes 5–19 cm in length; umbels 2–4-flowered; bracts 5, lanceolate, 5–10 mm long, 1–3 mm wide, membranous, base prolonged below into 3–6 mm auriculate appendage. Pedicel 0.5–3.5 cm. Calyx 5–7 mm in length, 3 mm diam., cylindrical, parted to 1/4 to 1/3; lobes lanceolate, apex narrowly acute. Corolla funnel-form, pink or purple; tube 8–10 mm in length, 8–10 mm diam., limb 1–2 cm diam.; lobes obovate, deeply emarginated apex.

Pin flowers: corolla tube ca. 8.5 mm; stamens ca. 3.5 mm above base of corolla tube; the style is not or slightly exerted.

Thrum flowers: corolla tube ca. 1 cm; stamens toward apex of corolla tube; the style is slightly shorter than calyx.

Capsule cylindric, apex irregular dehisces. (Fig. 2)

Distribution and habitat. Fissures of rocks on mountain slopes; ca. 2,800 m. Shennongding (Hongping, Shennongjia, Hubei), Laojunshan (Muyu, Shennongjia, Hubei). (Fig. 3)

Phenology. *Primula sunhangii* flowers from June to July.

Etymology. The specific epithet refers to the Chinese botanist Hang Sun.

Vernacular name: Simplified Chinese: 神农架报春; Chinese Pinyin: Shénnóngjià Bàochūn.

Conservation status and IUCN preliminary assessment. The new species has only two known populations described thus far. The populations are both in subareas of Shennongjia, one in Shennongding and another in Laojunshan. Each population has ca. 40 individuals. The habitats are situated in a tourist attraction zone, with an average of 20,000 tourists visiting daily. According to IUCN red list categories and criteria, conservation status of this species should be Critically Endangered (CR) (B2abiii).

Relationship with related species. Based on its glabrous, efarinose, ovate leaves, lanceolate bracts base prolonged below into auriculate appendage, and its

Figure 1. Holotype of *Primula sunhangii* T. Deng, D. G. Zhang & Jiao Sun, sp. nov.

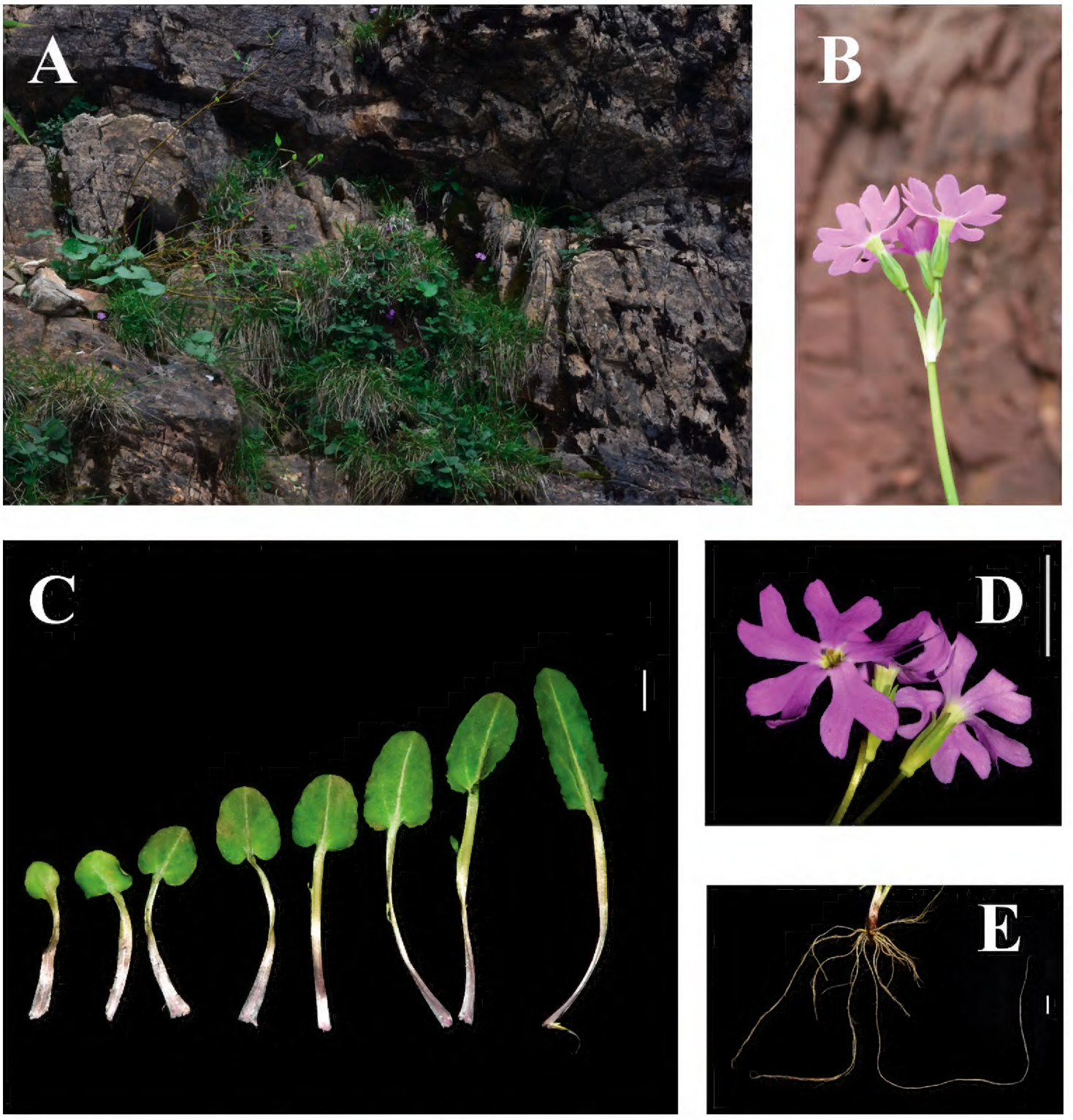

Figure 2. Images of live *Primula sunhangii* T. Deng, D. G. Zhang & Jiao Sun, sp. nov. **A** habitat **B** inflorescence **C** leaves **D** flower, front and back side **E** toot. Scale bars: 1 cm in (**C, D, E**). Photographer: Qun Liu.

length, *P. sunhangii* is most morphologically similar to *P. involucrata* (Hu 1990; Hu and Kelso 1996). But *P. sunhangii* differs from *P. involucrata* in glabrous sepal, cylindrical calyx and capsule, truncate or acute leaves base, and shorter scape (at most 3 times the leaves). (Table 1).

We performed phylogenetic analyses using nuclear ITS as well as *rbcL* and *matK* of *P. sunhangii* and related species. Separate analyses for all three markers get six trees (ML and Bayesian) with similar structures. Combined molecular phylogenetic analyses shows that *P. sunhangii* and *P. involucrata* are sister taxa, with high support (Fig. 4). They have 19 different sites in the 2020 bp nucleotide sequence.

These genetic results and morphological data clearly support that *Primula sunhangii* be recognized as a distinct new species.

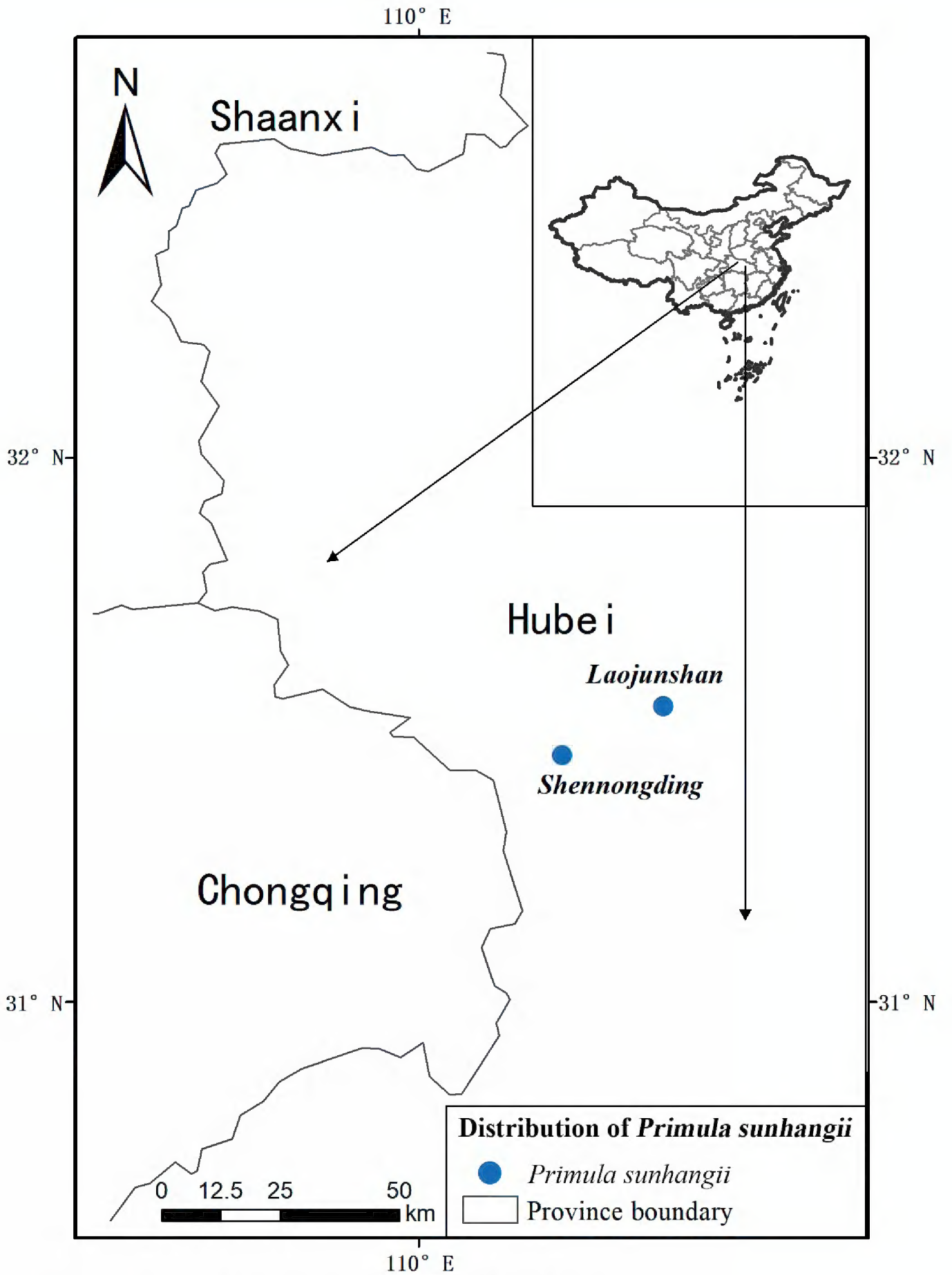

Figure 3. Distribution of *Prunus sunhangii* T. Deng, D. G. Zhang & Jiao Sun.

Table 1. Diagnostic morphological characters comparison between *Primula sunhangii* and *P. involucrata*.

Characteristics	*P. sunhangii*	*P. involucrata*
Base of leaves	truncate or acute	cuneate, rounded to slightly cordate
Sepal	glabrous	glandular ciliolate
Calyx shape	cylindrical	campanulate
Capsule shape	cylindrical	oblong
Ratio of scape to leaves	less than 3 times	3–5 times

Table 2. Primers used in PCR amplification and sequencing.

DNA fragments	Primer name	5'-3' Sequences
ITS	ITS1	GTCCACTGAACCTTATCATTTAG
	ITS4	TCCTCCGCTTATTGATATGC
rbcL	rbcLa_f	ATGTCACCACAAACAGAGACTAAAGC
	724R	TCGCATGTACCTGCAGTAGC
matK	3F-KIM	CGTACAGTACTTTTGTGTTTACGAG
	XF	TAATTTACGATCAATTCATTC
trnH-psbA	trnH-05	CGCGCATGGTGGATTCACAAATC
	psbA3	GTTATGCATGAACGTAATGCTC

Figure 4. Bayesian tree of selected species in *Primula*, focused on Sect. *Aleuritia*. Based on a combined sequences from nuclear ITS and *rbcL*, *matK* of cpDNA genes. Numbers above the branches represent the Bayesian posterior probabilities, and below showing the maximum likelihood values. (* multifurcation in Maximum-likelihood tree).

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Reference

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9(8): 772. https://doi.org/10.1038/nmeth.2109

Deng T, Zhang DG, Sun H (2018) Flora of Shennongjia (神农架植物志). China Forestry Publishing House, Beijing.

Hall T (2001) BioEdit: An important software for molecular biology. GERF Bulletin of Biosciences 2(1): 60–61. https://www.academia.edu/download/32793732/Bioedit_software_review.pdf

Hu CM (1990) Primula. In: Chen FH, Hu CM (Eds) Flora Reipublicae Popularis Sinicae, Chapter 59. Science Press, Beijing, 1–245.

Hu CM, Kelso S (1996) Primulaceae. In: Wu ZY, Raven PH (Eds) Flora of China. Science Press & Missouri Botanical Garden Press, Beijing & St. Louis.

Kusukawa N, Uemori T, Asada K, Kato I (1990) Rapid and reliable protocol for direct sequencing of material amplified by the polymerase chain reaction. BioTechniques 9(1): 66–68, 70, 72. https://europepmc.org/abstract/med/2393575

Li X, Bao D, Huang H, Xie J (2017) *Primula hubeiensis* (Primulaceae), a new species from central China. Novon: A Journal for Botanical Nomenclature 25(2): 162–165. https://doi.org/10.3417/2016032

Richards A (2002) Primula (2nd edn). B.T. Batsford, London.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics (Oxford, England) 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Wu X, Xu Y, Hu CM, Hao G (2013) *Primula mianyangensis* (Primulaceae), a new species from Sichuan, China. Phytotaxa 131(1): 49–52. https://doi.org/10.11646/phytotaxa.131.1.9

Xu Y, Liu TJ, Hu CM, Hao G (2015) *Primula anthemifolia* (Primulaceae), a new species from Sichuan, China. Annales Botanici Fennici 52(5–6): 373–376. https://doi.org/10.5735/085.052.0518

Xu Y, Yu XL, Hu CM, Hao G (2016) Morphological and molecular phylogenetic data reveal a new species of *Primula* (Primulaceae) from Hunan, China. PLoS One 11(8): e0161172. https://doi.org/10.1371/journal.pone.0161172

Xu Y, Huang G, Hu C, Hao G (2017) *Primula pengzhouensis* (Primulaceae), a new species from Sichuan, southwestern China. Plant diversity 39(4): 229–231. https://doi.org/10.1016/j.pld.2017.08.003

Yan HF, Liu YJ, Xie XF, Zhang CY, Hu CM, Hao G, Ge XJ (2015) DNA barcoding evaluation and its taxonomic implications in the species-rich genus *Primula* L. in China. PLoS One 10(4): e0122903. https://doi.org/10.1371/journal.pone.0122903

Supplementary material 1

Taxa, specimen collection information, GenBank accession numbers of all studied samples

Author: Jiao Sun

Data type: species data

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